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OSHA-79-1750

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Arkh. Patol. 35(11):67-73, 1973 ?

Arkhiv Patologii

RUSSIAN

Arch. Pathol. 35(1):67-73, 1973 USSR

EXPERIMENTAL HISTOLOGICAL INVESTIGATION OF PATHOMORPHOLOGY OF THE MYOCARDIUM IN CHRONIC CHROMIUM INTOXICATION.

[Article by A. M. Shakhnazarov]

Published by the Department of Pathological Anatomy (Director: S. I. Alkadarskii, M. D.) and the Department of Pharmacology (Director: Professor V. M. Senikov).

Summary

Experimental studies of the myocardium with the use of morphological, histochemical nd histoenzymochemical methods in 5 control and 38 experimental dogs, which during he period of 1—3 years received enterogenously sodium bichromate in doses oi t. 3. 5. 0 mg per 1 kg of body weight, were carried out. The studies showed that prolonged peroral intake oi bichromate in small doses (1 mg/kg) brought about a total decrease oi he glycogen level. focal inhibition of the activity of reduction-oxidation and hydrolytic nzymes, a drop in the content of SH-groups and in that oi ascorbic acid, without noticable histological changes. With the dose of 10 mg/kg there were noticed, against the ackground of parenchymatous dystrophy, a drastic decrease in the glycogen content not nly in the contractile myocardium, but in Purkinje iibres as well, more expressed inhiibition oi the enzymes under study. It the same time a high content of acid phosphatase nd RNA in the histogenic elements oi the stroma and a high activity of monoaminoxidase n the muscular iibres were also noted. The damage of the blood vessels may account for he absence of a full therapeutic effect oi unitiol and for the progressing oi pathological hanges in organs after the discontinuance of the contact cith chrome in chronic chromic ntoxication.

Clinical studies have demonstrated that the cardiovascular system suffers frequent damage in persons who have contact with the chromium salts (L. N. Belyaefa, A. M. Kleiner et al., T. A. Shakirov, Gafafer). The results of numerous experimental studies demonstrated the marked functional and morphological deviations of the heart following exposure to the chromium compounds (Ya. M. Grushko; E. F. Faidysh, V. P. Ershov, V. P. Ershov and V. A. Fedorova, A. M. Kryshtab; Fritz et al.).

Most of the published studies on myocardial toxicity in chromium poisoning are limited to descriptions of the histological changes in the myocardium; some of the individual studies report on the amount of glycogen, RNA and ascorbic acid (V. A. Yaglinskii and A. M. Shabanov, M. G. Spasskaya et al., S. N. Gordina and V. A. Yaglinskii). However, the more detailed and

pronounced biochemical changes at the cellular and tissue level still remain little studied in chromium toxicity. In view of these deficiencies, we decided to study the condition of the myocardium after prolonged oral administration of various doses of sodium bichromate in an aqueous solution, and to apply various histochemical and histoenzymological methods.

The material for the study consisted of the hearts of 58 experimental and five control dogs of both sexes between one and two years of age and weighing between 10 and 15 kg. All the animals were divided into four groups. Group one included the animals who were given oral doses of sodium bichromate in amounts of 1, 3, 5 and 10 mg/kg for 1, 1.5, 2 and 3 years (a total of 48 dogs, three dogs tested for each time period). In the animals of the second group, we studied the reversible changes one year after stopping the administration of chromium. This group consisted of five dogs given daily, increasing doses of sodium bichromate for two years (1 mg/kg for six months; 3 mg/kg for one year; 5 mg/kg for six months). In the animals of the third group (five dogs), we studied the organs after two years of toxicity with bichromate in doses of 10 mg/kg and a 15 day course of detoxifying therapy consisting of unithiol. The fourth group (controls) consisted of five dogs, maintained under identical conditions to thoseof the experimental animals and observed along with the latter. The animals were sacrificed by blood letting from the cervical artery after being given a stunning blow. From the right and left ventricle of the beating heart, we cut out fragments some of which were immediately immersed and fixed in Shabadash and Carnua stains. For the histological and enzymological studies, the sections having a thickness of 10 microns were prepared in a cryostat. In the freshly frozen section, we determined cytochrome oxidase using the method by Greff, succinate dehydrogenase

using the methods by Nakhlas and Lilly, NAD–diaphorase using the method by Scarpelli, Gess, Pears, monoamine oxidase using the method by Glenner with nitro–T electron acceptors. In the sections fixed in cold formalin and calcium mixtures (Erenke), we determined the activity of alkaline and acid phosphatase using the method by Gomor, adenosine triphosphatase (ATP–ase) using the method by Wachstein and Meisel, acetyl cholinesterase using the method by Koelle and Lipid Sudan III + IV and Sudan black B. For the ascorbic acid, the material was processed according to the Ciroud and Leblond method. The paraffin sections were stained by the Shabadash method for glycogen, the Bariett and Seligman method for the sulfhydryl groups (SH–groups), the Brash method for RNA, the Stidmen alcian blue method and for colloidal iron, the Hale method on acid mucopolyssacharides, were used as well as hematoxylin eosin and picrofuchsin. The histochemical methods were those of A. L. Shabadash, Pierse, Berston, Lilly and Kisel.

In the intact animals, the myocardium contained a significant amount of glycogen, SH groups, succinate dehydrogenase, cytochrome oxidase and NAD–diaphorase, moderate monoamine oxidase activity and acid phosphatase and slight pyroninophilia. The activity of alkaline phosphatase was good in the capillary endothelium. ATPase activity in the walls of the arterioles and capillaries was good as was acetylcholinesterase in the nerve fibers and in the Purkinje fibers.

One to two years after the start of the bichromate toxicity in a dose of 1 mg/kg, no histochemical changes were detected. The amount of glycogen, SH groups and ascorbic acid in the muscle fibers was reduced. Three years of bichromate administration produced morphologically thickening of the capillary walls, and in the arterioles and arteries of moderate diameter. In some of these, we found a proliferation of the endothelial and

adventitial cells, a moderate growth around the vessels of the fibrillar connective tissue. In the individual fields of vision, we found sites of myofibrosis with atrophy of the muscle fibers, a thickening of the connective tissue in the endomysium and perimysium, and in the sarcolemma. The total decrease in the glycogen level (Figure 1) and in ascorbic acid was accompanied by local reductions of the SH-groups, cytochrome oxidase and succinate dehydrogenase in the parenchyma (Figure 2). The activity of acetylcholinesterase, alkaline phosphatase and ATP-ase was markedly more irregular; there were segments with low activity associated with fragments having elevated and diffuse staining of the sarcoplasma (Figure 3). The activity of monoamine oxidase was generally elevated; only in individual fields did we encounter small foci with rare depositions of formasan granules. In the stroma, the amount of fibroblasts was increased as was the number of plasmatic cells with large amounts of RNA and acidphosphatase. The amount of acid mucopolysaccharides was somewhat increased in the intramural blood vessels and in their surrounding connective tissue.

In studying the myocardium of animals given sodium bichromate in a dose of 3 mg/kg for one year, we failed to observe any definite histological changes. Histochemically, we found sites showing a decrease in the amount of glycogen and ascorbic acid in the muscle fibers of the middle and subepicardial zones. After two and especially after three years, we noted a definite thickening in the basal membrane of the capillaries, moderate sclerosis of the arterioles and perivascular connective tissue. At the same time, there was a total reduction in the amount of glycogen and ascorbic acid in the muscle fibers combined with a segmentary decrease in the SH-groups, succinate dehydrogenase, cytochrome oxidase, NAD-diaphorase; the activity of alkaline phosphatase and ATP-ase in the capillary walls was markedly more irregular than in controls.

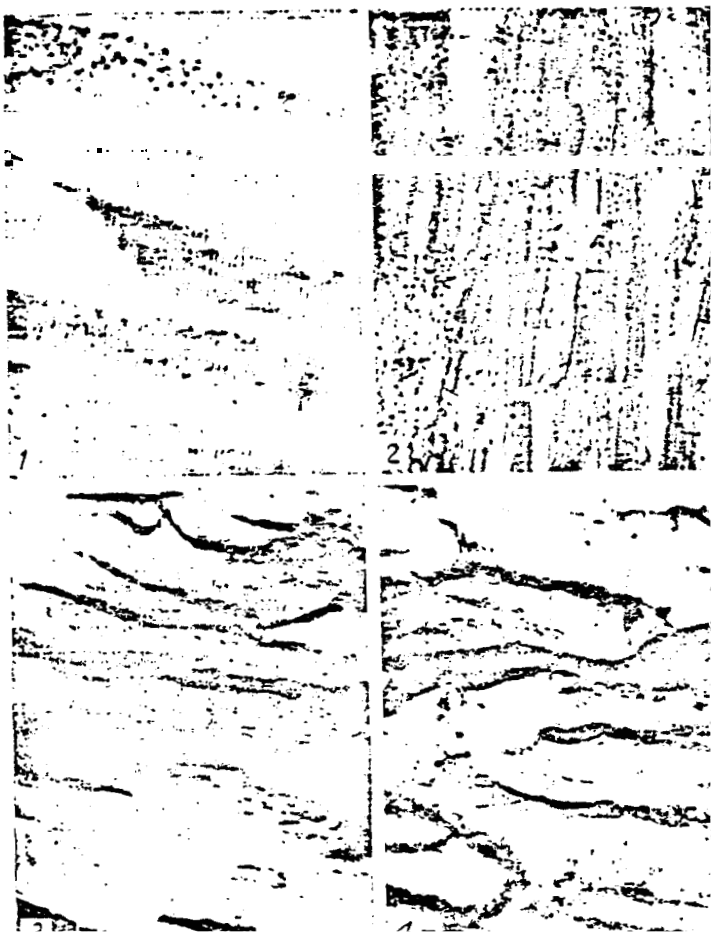


Figure 1. Marked decrease of glycogen in the muscle fibers with the preservation of cross striations during the daily oral administration of sodium bichromate for three years. PAS reaction X *400.*

Figure 2. Foci of reduced succinate dehydrogenase activity in the muscle fibers with a deposition of non-oriented consolidated granules of formasan during the administration of sodium bichromate for two years. Nakhlas method with nitro-ST X *400.*

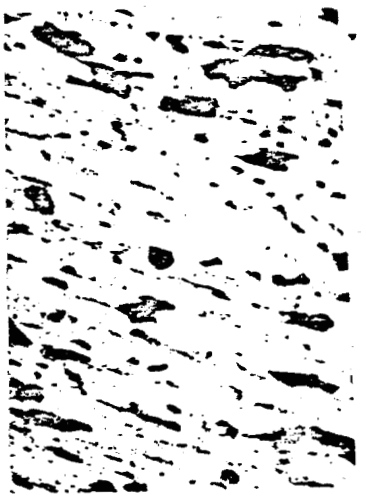
Figure 3. Irregular activity of alkaline phosphatase in the capillary walls with diffusion into the muscle fibers after three years of sodium bichromate administration. Gomor's reaction X *400.*

Figure 4. Irregular activity of ATPase in the capillary walls with a segmentary disappearance after two years of sodium bichromate administration. Wachstein and Meisel method X *400.*

As in the animals of the previously discussed group, when a 5 mg/kg dose of sodium bichromate was administered for one year, no marked morphological changes were found. Histochemical examinations only revealed a significant decrease in the amount of glycogen and SH groups in the muscle fibers. After three years, we observed small foci of myofibrosis, a segmentary thickening of the basal membrane of the capillaries in arteriolar sclerosis. The muscle fibers were reduced in these portions (see Figure 3) while the amount of ascorbic acid was increased in the vascular walls. An inhibition in the total activity of acetylcholinesterase was associated with normal activity of monoamine oxidase. The activity of the oxidative reduction enzymes generally remained high and only in some sites did we encounter small foci of oxidative reduction inhibition. The activity of alkaline phosphatase in the capillary walls was irregular (Figure 4) and the acid phosphatase was irregularly distributed in the muscle fibers being somewhat higher in the stromal histiocytes, as was also the amount of RNA (Figure 5).

Figure 5.

Marked irregular activity of acid phosphatase in the muscle fibers after two years of toxicity with sodium bichromate. Gomor's reaction X 250.



In the animals given a 10 mg/kg dose of sodium bichromate, the myocardium contained more marked histological and histochemical changes than in the animals of the previous groups. The dogs sacrificed after 1, 1.5 and 2 years showed granular dystrophy of the myocardium. In the peri-nuclear zones of the muscle fibers, we found fiber granules having an affinity for Sudan dye (Sudan Black B). The amount of glycogen in the muscle fibers of the myocardium was markedly reduced, especially in dogs sacrificed after two years. In the latter animals, there was a depletion of glycogen in the Purkinje fibers. Glycogen was present in the sarcolemma in the form of small linear accumulations of the smallest: granules. In many of the capillary loops, there was inhibition of ATPase and alkaline phosphatase. In the animals of this group, there was a definite reduction in the amount of SH groups and ascorbic acid in the muscle fibers. The activity of acetylcholinesterase was definitely inhibited in all the cholinergic structures after one, and especially after two years, while at the same time, the activity of monoamine oxidase showed no particular change. As for the cytochrome oxidase, succinate dehydrogenase and NAD-diaphorase, we observed a general decrease in their activity with small sites of significant inhibition and disorders in the orientation of the parallel myofibrils.

In the animals given sodium bichromate for a two-year period in increasing doses (one mg/kg for six months; 3 mg/kg for one year; 5 mg/kg for six months; for a total of 2 g and 150 mg) and sacrificed 1 year after the introduction of the metal was stopped (second group), in the myocardium we found foci of atrophy and dystrophic processes against a background of moderately evident myofibrosis and sclerosis of the small branch of the

coronary artery. The muscle fibers contained a definite reduction in the SH groups, glycogen and ascorbic acid; the activity of the oxidative reduction enzymes was reduced in some sites.

In the animals of the third group subjected to chronic chromium toxicity for two years and given doses of 10 mg/kg with a subsequent 15 day courses of therapy with unithiol (10 mg/kg), we found (by using Sudan Black B) foci of parenchymatous dystrophy in the muscle fibers with deposits of small droplets of lipids. In these segments, the amount of all the studied enzymes was reduced. In the capillary walls, there was a slight increase in the activity of ATPase.

In the animals of the various groups, at different periods (first group: 1 mg/kg; three years - 2 cases; 3 mg/kg, 2 and 3 years; one case; 5 mg/kg, 3 years - 2 cases; 10 mg/kg, 2 years - 2 cases; second group: 3 cases; third group: 2 cases), we found within the intramural arteries of the heart "kidney-like'' growths of the endothelial cells and segments with concentric or lamellar sclerotic thickenings of the intima (Figure 6).

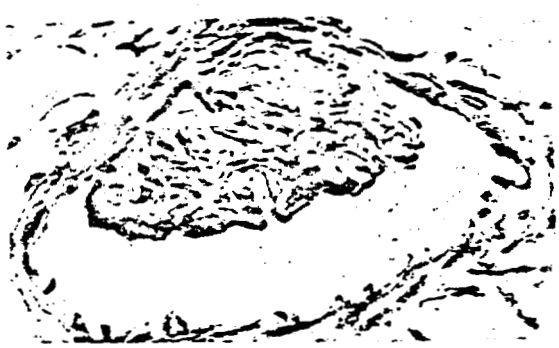


Figure 6. Lamellar thickenings of the vascular intima in dogs, administered sodium bichromate in increasing doses: 1 mg/kg for six months; 3 mg/kg for one year; 5 mg/kg for six months (total of two grams and 160 mg). Stained with hematoxylin eoxin X 400.

Thus, in the myocardium of dogs given oral chronic administration of small, moderate and toxic doses of sodium bichromate, we found certain histological and histochemical changes. These changes are indicative of focal disorders in the metabolic processes in the various groups of muscle fibers, during the course of individual histochemical changes leading to the development of histological modifications.

It is possible that the toxic activity of bichromate after prolonged administration is related to its direct effects on the myocardial parenchyma during the early stages of toxicity and depends on its association with a vascular component (hypoxia) at a later stage. The studies by O. M. Shabanov and L. M. Pamurzin demonstrated an accumulation of chromium in the cardiac tissue after experimental burns on the skin with bichromate. The vascular damage can be explained by the fact, that stopping the bichromate administration or subsequent therapy with unithiol failed to produce complete normal recovery of the myocardium after chronic toxicity with sodium bichromate in moderate and toxic doses. L. N. Belyaeva noted the possibility of progressive pathological changes in the organs after the discontinuation of contact with chromium.

The prolonged administration of bichromate in a dose of 10 mg/kg produced a marked reduction in glycogen not only in the contractile myocardium but also in the Purkinje fibers, where the relatively stable amount of polysaccharide can be indicative of the toxic activity of bichromate as well as myocardial hypoxia with increased glycolysis. The diffuse reaction of alkaline phosphatase around the vascular capillaries and its focal distribution in the muscle fibers, and irregular distribution of the acid mucopolysaccharides in the basal membranes of the capillaries and the segmentary decrease in the activity of ATPase in the latter are

indicative of disorders in the permeability of the intramural walls of the cardiac vessels. The accumulation of RNA in the cytoplasm of the epithelial and plasmatic cells, fibroblasts and capillary endothelium and the increased activity of acid phosphatase demonstrate irritation to the mesenchyma and an elevation of the metabolic and synthesizing processes.

The elevated activity of monoamine oxidase can be explained by an increase in the metabolism of biogenic amines in the myocardium (epinephrine, norepinephrine, dopamine, etc.), which appear to be substrates of monamine oxidase. The activity of acetylcholinesterase, in response to the metabolism of the parasympathetic mediator acetylcholine, was inhibited in the nerve fibers, and in the conducting fibers of the heart and in the muscle fibers during the chronic administration of small, moderate, large, and toxic doses of bichromate. These findings agree with the pharmacological and physiological studies (V. M. Sennikov and M. K. Naumova) on the basis of which the hypothesis regarding the "m- and n-cholinomimetic" mechanism of activity of hexavalent chromium in the body, was proposed.

RESULTS

1. Histochemical studies demonstrated slight biochemical changes in the myocardium leading to the development of histological modifications after the oral administration of sodium bichromate in various doses (1, 3, 5 and 10 mg/kg).

2. The pathogenetic effects of sodium bichromate on the heart after prolonged administration to the organism are related not only to its direct effects on the myocardial parenchyma but also are carried out via the vascular system.

3. The focal reduction in the activity of oxidative reduction enzymes and hydrolytic enzymes in the myocardium, the inhibition of cholinesterase during elevated activity of monoamine oxidase, the decrease of glycogen in the contractile myocardium and in the Purkinje fibers may be basically related to the functional deviations in the cardiovascular system observed in workers during chronic poisoning with chromium.

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